

E6-AP siRNA (m): sc-40682

BACKGROUND

E6-associating protein (E6-AP), also designated ubiquitin protein ligase E3A (UBE3A), is a component of the ubiquitin-mediated proteolytic pathway that selectively targets proteins for degradation by the 26S proteasome. Ubiquitin (Ub) is directly conjugated to protein substrates by the transfer of Ub from an E2 ubiquitin conjugating enzyme to the target protein. This conjugation is facilitated by the enzymatic activity of E3 ubiquitin ligase family members such as E6-AP. Several substrates of E6-AP have been identified and include the tumor suppressor protein p53 and the mammalian homolog of Rad23, HHR23A. Previous studies have indicated that E6-AP associates with the human papillomavirus E6 oncogene, which forms a complex with p53 and thereby potentiates E6-AP mediated ubiquitination of p53. Genetic mutations that impair E6-AP activity result in the accumulation of p53 in the cytoplasm, and in many instances, these mutations are associated with the development of the rare neurodevelopmental disorder Angelman syndrome (AS), which is characterized by severe motor dysfunction and mental retardation.

REFERENCES

1. Jentsch, S. 1992. The ubiquitin-conjugation system. *Annu. Rev. Genet.* 26: 179-207.
2. Huibregtse, J.M., et al. 1993. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. *Mol. Cell. Biol.* 13: 775-784.
3. Yamamoto, Y., et al. 1997. The human E6-AP gene (UBE3A) encodes three potential protein isoforms generated by differential splicing. *Genomics* 41: 263-266.

CHROMOSOMAL LOCATION

Genetic locus: Ube3a (mouse) mapping to 7 C.

PRODUCT

E6-AP siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see E6-AP shRNA Plasmid (m): sc-40682-SH and E6-AP shRNA (m) Lentiviral Particles: sc-40682-V as alternate gene silencing products.

For independent verification of E6-AP (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40682A, sc-40682B and sc-40682C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

E6-AP siRNA (m) is recommended for the inhibition of E6-AP expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

E6-AP (E-4): sc-166689 is recommended as a control antibody for monitoring of E6-AP gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor E6-AP gene expression knockdown using RT-PCR Primer: E6-AP (m)-PR: sc-40682-PR (20 μ l, 525 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kilroy, G., et al. 2009. High efficiency lipid-based siRNA transfection of adipocytes in suspension. *PLoS ONE* 4: e6940.
2. Mishra, A., et al. 2013. E6-AP association promotes SOD1 aggregates degradation and suppresses toxicity. *Neurobiol. Aging* 34: 1310.e11-23.
3. Kumar, V., et al. 2019. Simvastatin restores HDAC1/2 activity and improves behavioral deficits in Angelman syndrome model mouse. *Front. Mol. Neurosci.* 12: 289.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.